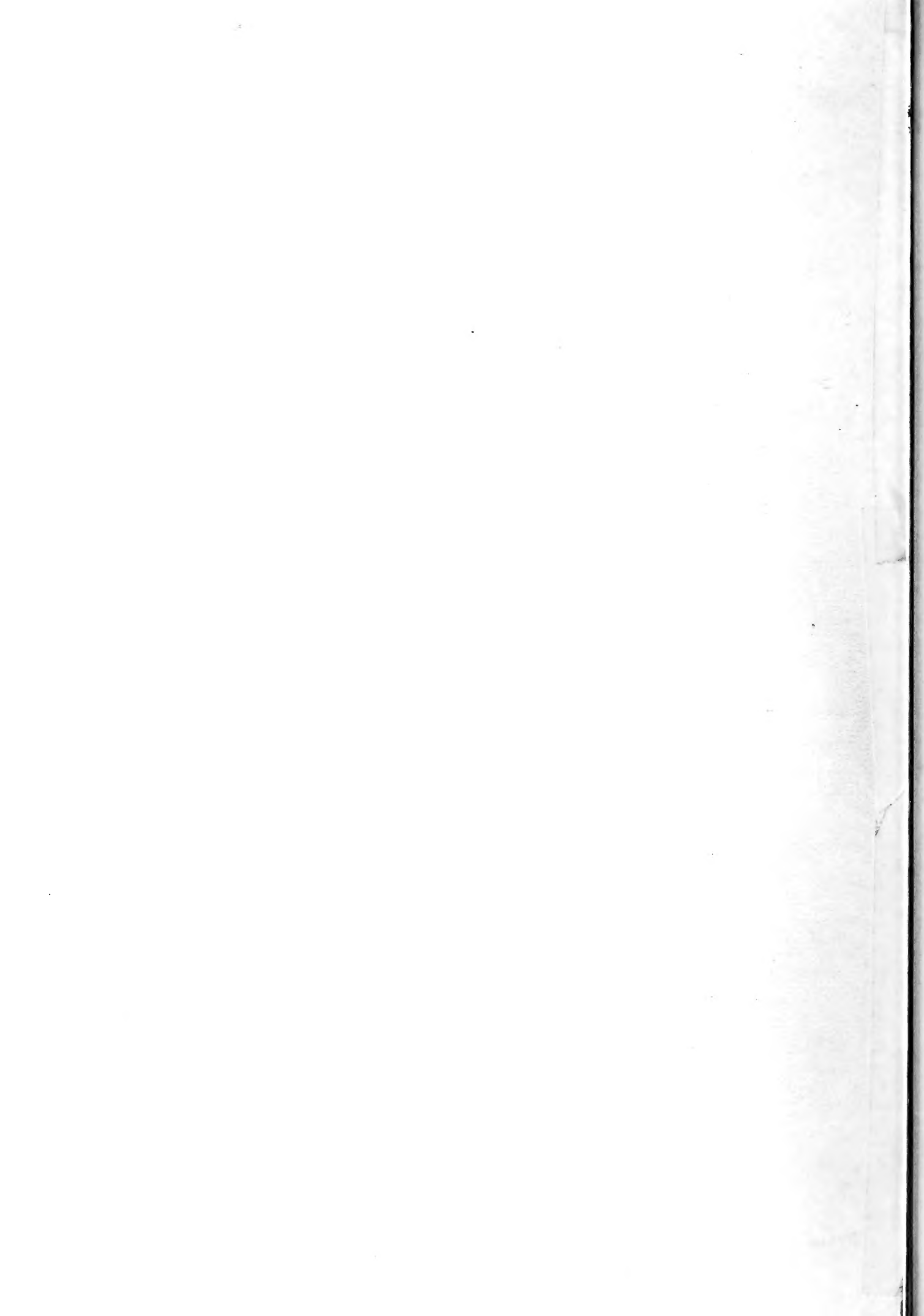


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United States Department of Agriculture  
Bureau of Entomology and Plant Quarantine

A TECHNIQUE FOR THE RECOVERY OF VERY SMALL  
DEAD INSECTS IN MORTALITY EXPERIMENTS

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Insect Investigations

The apparatus and methods described were developed in connection with studies of the resistance of sugar beets to the beet leafhopper. In these experiments a given number of females were allowed to oviposit in a single leaf for a given length of time. For the greatest efficiency in experimentation, it was necessary to determine accurately the total hatch of nymphs and the mortality of these nymphs from the first instar to maturity. This required not only the recovery of live insects at the end of the experiment but also all dead individuals. Some of the latter died in the first instar, and leafhoppers which die in this stage shrivel to a mere speck, impossible to find in any ordinary cage by the usual methods. All known types of leaf cages small enough to permit recovery of dead leafhoppers cause death of the leaf after a few days because they depend on pressure for attachment to the leaf. This is a serious disadvantage in long experiments.

Drawings and dimensions of the cage that was developed to overcome these difficulties are shown in figure 1. The top and bottom are made of fine-meshed muslin cloth, the sides and ends of celluloid. There is a round hole  $7/16$  inch in diameter in one end of the cage and another hole  $13/16$  inch in diameter in the other end. Figure 2 shows construction details of a wire support for the cage. The method of mounting the cage on potted beet plants is illustrated in figure 3.

The first step in mounting is the wrapping of a band of cotton around the leaf petiole to form the cotton plug. Unwrapping of the band is prevented by twisting a few strands together with the fingers. The cotton should be a good grade of batting of the more springy type, that is, having stiff fibers. The common absorbent cotton tends to lose its resilience if it becomes wet when the plants are watered. Enough cotton should be used to close firmly the opening around the petiole but not enough to cause more pressure than is necessary.

After the cotton, the wire support is put in place by pushing the ends of the wire into the soil. Then the leaf, which is usually wider than the large hole in the cage, is gently folded or rolled, and the cage is pushed over it and down around the cotton plug. A cork stopper closes the small upper hole in the cage.

If the bottom of the cage rests on the damp soil of the pot, it is well to put a small piece of wood under it as a support. Otherwise the cloth will rot in a short time.

Insects can be put into the cage or removed from it with a glass pipette through the small upper opening. If the pipette is only slightly smaller than the hole in the cage, insects cannot escape past it during these manipulations.

At the end of the experiment living insects are removed and counted. The petiole can be cut below the cotton plug to facilitate handling and permit shaking of the cage to make sure that all live insects are taken out. Dead insects are then removed with the apparatus diagramed in figure 4. The rubber tube shown on the right of the figure is attached to a machine that produces a strong suction. The long glass tube at the top of the figure is inserted into the cage and the cage is carefully vacuum cleaned. The cotton plug and the leaf are usually removed over a piece of paper and cleaned outside the cage. All dead insects, cast skins, and dirt are deposited on the cloth filter. This filter is then removed and the insects are sorted and counted under the microscope.

The data at the end of the experiment include total live insects and total dead in each instar. From these data the total hatch and the percentage of mortality are calculated.

Various tests have shown this technique to give highly accurate results provided a strong enough suction is used to pick up all insects and provided the suction tubes, cage, and leaf are dry.

In some experiments the leaf surface becomes very sticky from leafhopper excrement or the exudation of sap from feeding punctures. Dead leafhoppers may become stuck so tightly that they are not picked up by the suction machine. This can be prevented by soaking the cages with their contained leaves and insects in water for about an hour and then drying. The sticky substance is dissolved.

After live insects are removed from the cages, if the cleaning process is delayed as may be necessary in large experiments, the leaves may become so dry and brittle that they break into small pieces when cleaned. This debris is picked up by the suction machine and mixed with the dead insects, which makes sorting a very

laborious job. This can be avoided by placing the cages in a moist relaxing jar for an hour or more before cleaning. If they are left in the jar more than a few hours, a spoonful of formaldehyde should be added to the water to prevent molding.

If the cages become dirty, they can be washed in water and scrubbed inside with a bottle brush. With continued use the cages may become warped and misshapen. Most of this deformity can be corrected by drying them, after washing, on the spreader shown in figure 5. The spreader is inserted into the cage by pressing the two short arms together until the long arms can pass through the large hole in the cage. The spring is then released, allowing the long arms to press outward on the celluloid sides of the cage, forcing it into approximately its original shape.

#### Method of Constructing the Cage

First prepare a wooden form. Using a coping saw, cut from a 1-inch board a plug the size and shape of the cloth top of the cage. Enlarge the saw cut so that one thickness of cloth and celluloid can be held tightly between the plug and the form when the plug is in the hole from which it was cut.

Using the wooden plug as a pattern, mark the cloth with a soft pencil and cut  $1/8$  inch larger than the pattern on each side. If it is desired to number the cages, a numbering machine that stamps consecutive figures can be used to print numbers on the cloth.

The celluloid is cut into strips  $1-1/2$  inches wide and 19 inches long. The holes are cut by clamping one or more pieces of celluloid between wooden blocks and boring through with a carpenter's bit.

To assemble the cage, place the wooden form flat on the table with the plug out. Put one of the pieces of cloth, which are to form the sides of the cage, flat in the bottom of the hole with the edges turned up evenly along the sides of the form. Then place the celluloid strip on edge around the inside of the form and inside the turned-up edges of the cloth. Now force the wooden plug into place inside the celluloid. Cloth and celluloid will now be held firmly in place. Cement the overlapping ends of the celluloid strip together with acetone. Turn the form over and cement the cloth to the celluloid by allowing a little acetone to run down the crack between the wooden plug and the form.

Remove the wooden plug and the partly completed cage from the form and place the second piece of cloth in the bottom of the form as before. Fold the cage slightly and put the unfinished side

down in the hole in the form. It will now expand sufficiently to hold the cloth in place without the wooden plug. Turn the form over and cement the cloth to the celluloid. Remove the cage from the form and stick down the cloth in any place where it is not firmly attached. If the cloth does not lap over the celluloid more than 1/8 inch, any wrinkles along the edge that is cemented to the celluloid can be worked out by moistening the cloth with acetone and smoothing with the fingers.

#### Construction of the Suction Apparatus

All the connections of this apparatus (fig. 4) must be made airtight. The large rubber stopper, which is set into the wooden block, should be firmly cemented to the block. The curved end of the long glass tube greatly facilitates cleaning cages, since corners and the less accessible parts of the cage can be reached with it. The glass tube that fits into the large rubber stopper and holds the cloth filter in place is made larger than the other tubes to provide a larger area of filter. As a result of this construction, a strong current of air passes through the small tubes, preventing insects from becoming lodged, and a weaker current passes through the filter without forcing the insects through the cloth.

#### The Effect of the Cage on Physical Factors

When these cages were put on beet leaves in the greenhouse, the temperature inside the cages was usually lower than outside. This difference increased as the temperature outside became higher. For instance, at 80° F. the difference was approximately 2°; at 90° the difference was about 5 degrees. When outside air was forced into the cage through a tube, the difference was still greater, being under our conditions 4° at 80° and 9.5° at 90°. This suggests that these temperature differences are due to evaporation from the leaf, since a current of air in the cage would increase evaporation.

No measurements of light have been taken inside the cages, but these cages appear to be well lighted. Some beet leaves inside the cages have survived as long as 8 weeks in excellent condition. Other leaves have turned yellow and died within 2 or 3 weeks under conditions where they would normally have lived a much longer time outside. Lower light intensities inside the cages probably are a contributing cause of this abnormal behavior.

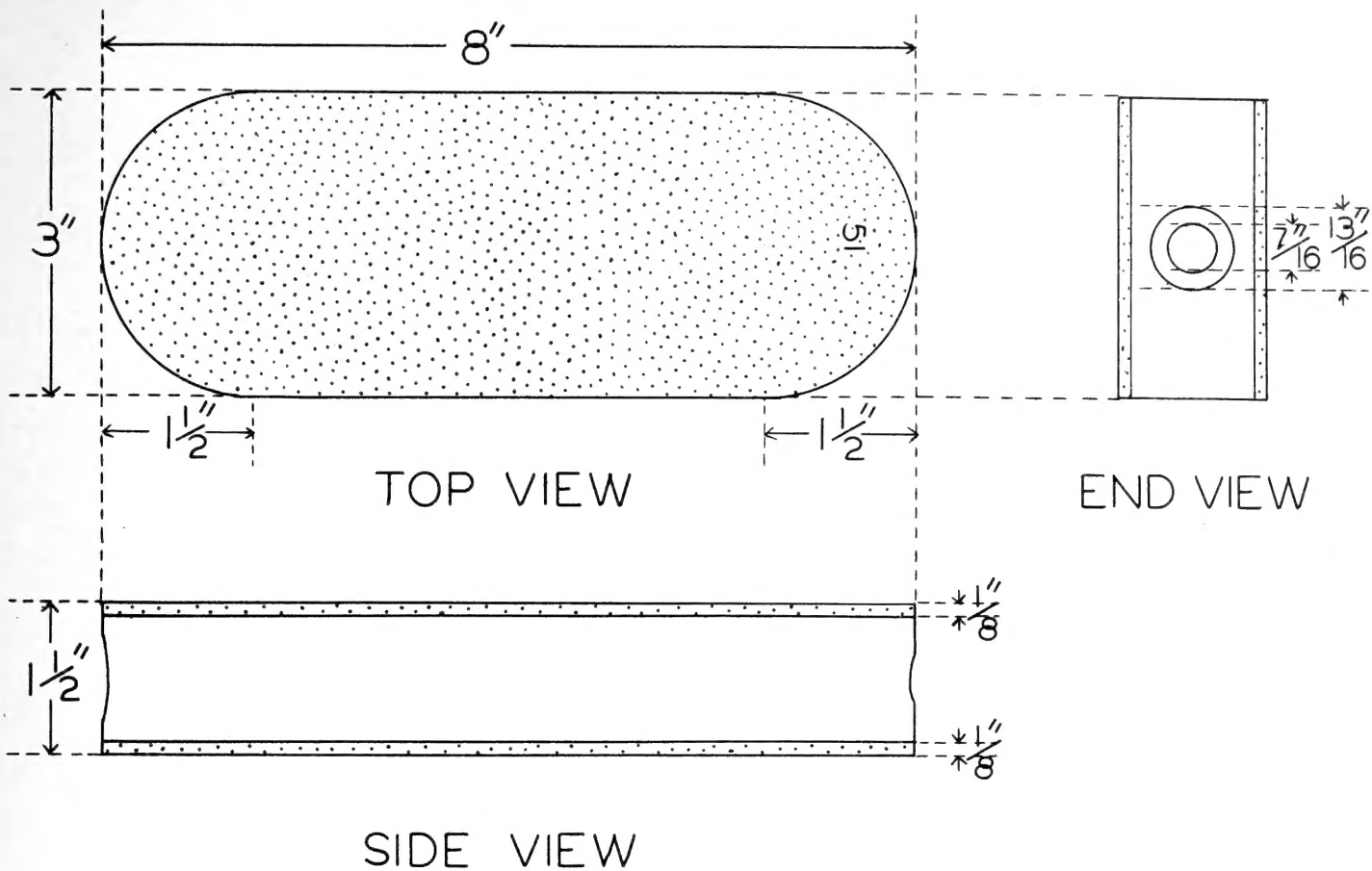


Figure 1.—The cage used. Stippled parts of the drawing represent close-meshed muslin cloth.

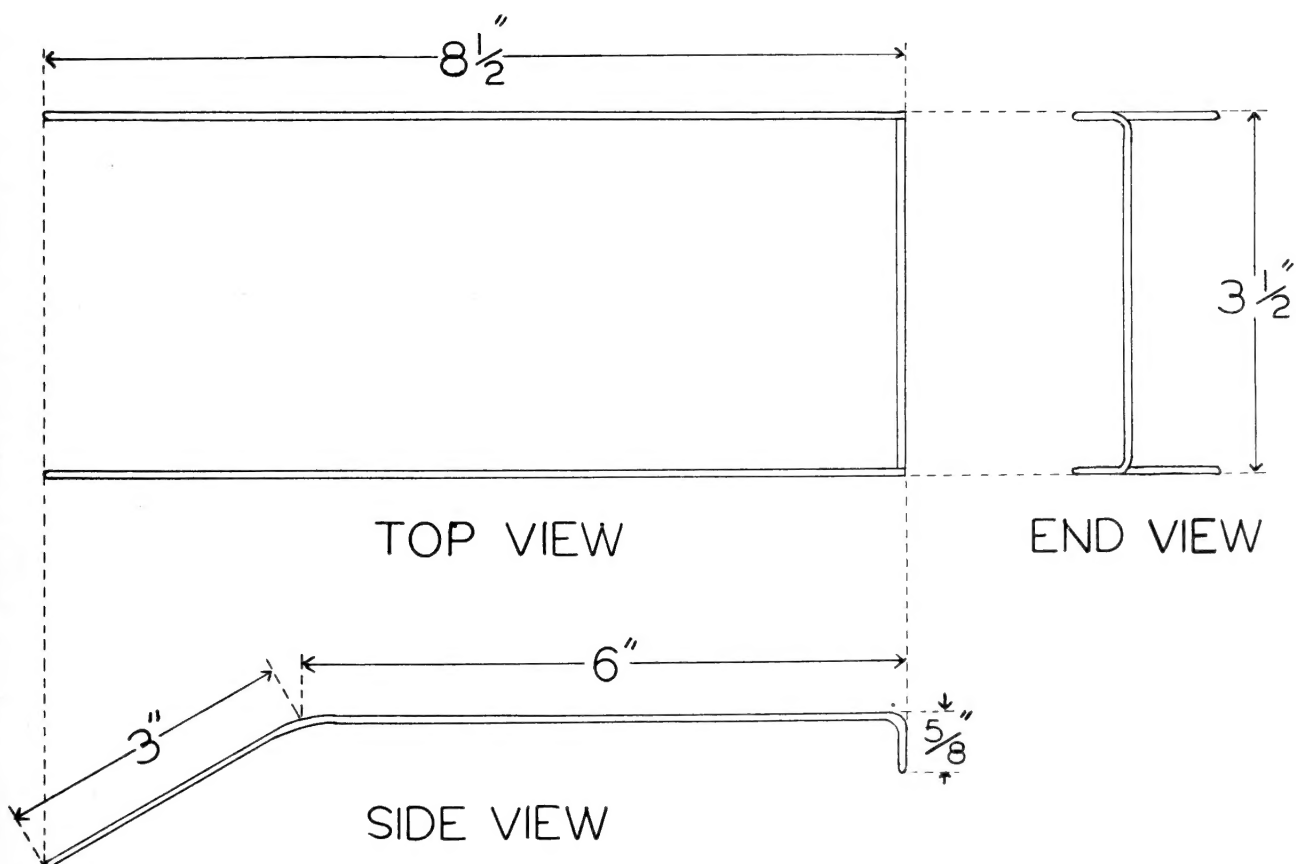


Figure 2.—Wire frame used to support the cage. The dimensions need not be exact.





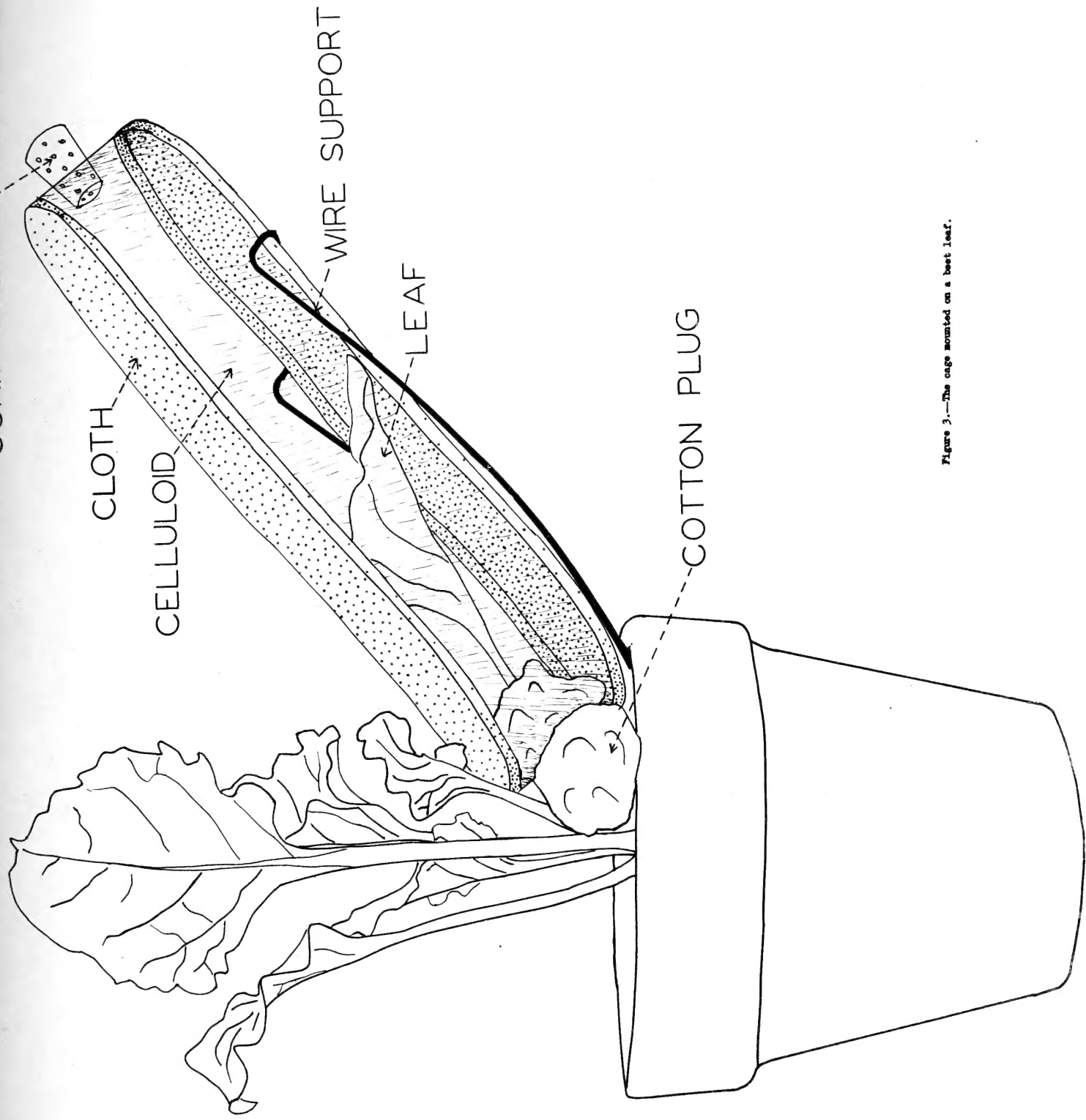


Figure 3.—The cage mounted on a beet leaf.



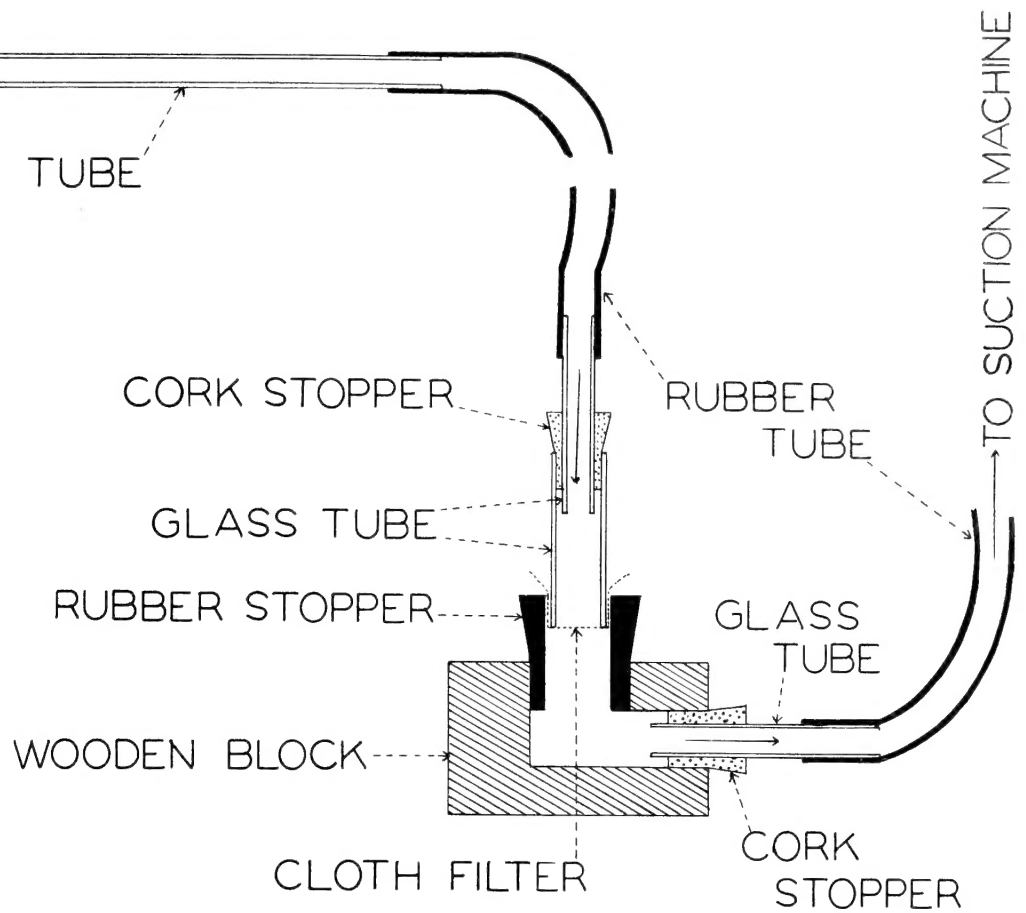


Figure 4.—Diagrammatic cross section of apparatus used in removing dead leafhoppers from the cogen. The arrows indicate direction of air current.

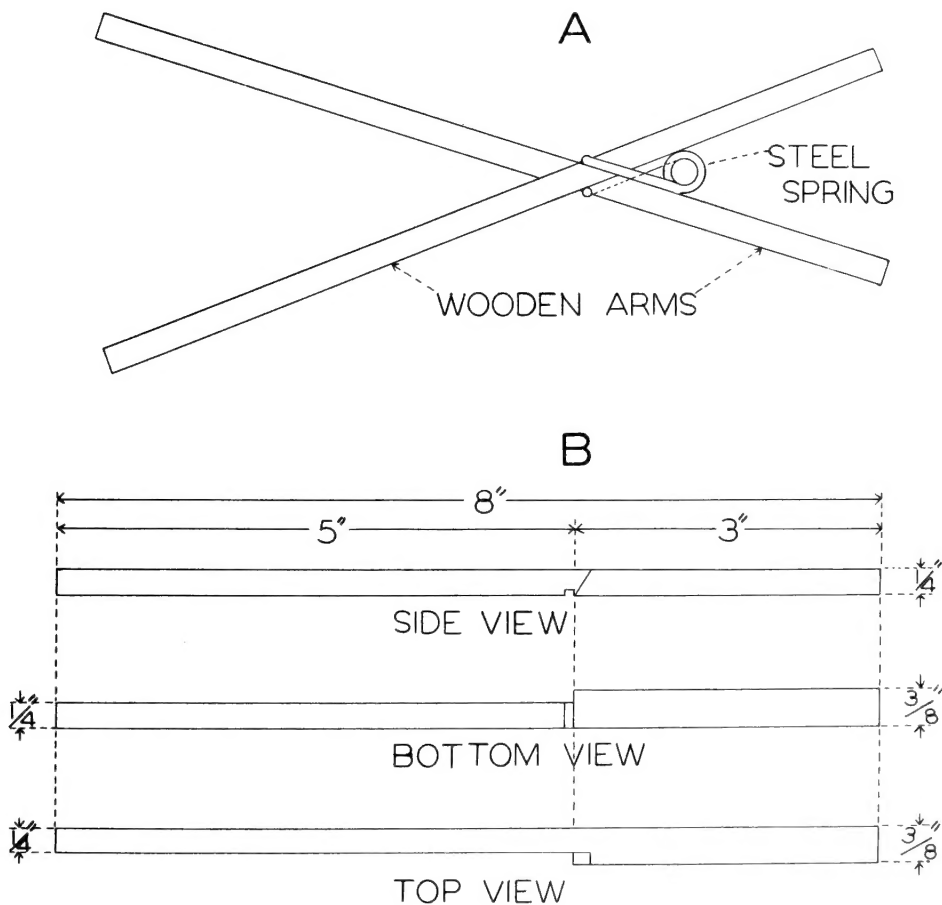


Figure 5.—(A) Spreader on which cages are dried after washing. The steel spring is from an ordinary clothespin and is not modified.  
(B) Detailed drawing of a wooden arm. Both arms are made alike.

